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Impact of Stress on Longevity: A Study in Two Races of *Immigrans* Species Subgroup of *Drosophila*

[1]*Ramesh Babu Yarajarla and* [2]*B.P. Harini*

[1]Centre for Applied Genetics, Jnana Bharathi, Bangalore University, Bangalore-560 056, India
[2]Drosophila Culture Laboratory, Department of Zoology, Jnana Bharathi,
Bangalore University, Bangalore-560 056, India

Abstract: *Drosophila nasuta nasuta* (2n = 8) and *Drpsophila nasuta albomicans* (2n = 6) are a pair of allopatric sibling species, belonging to the *nasuta* sub-group of the *Drosophila immigrans* species group. These two siblings are morphologically almost identical and are cross-fertile, but they differ with respect to their karyotypic composition. Hence, they are termed as chromosomal races. In the present study allopatric races of *nasuta* subgroup of *Drosophila* namely *D. n. nasuta* and *D. n. albomicans* were analyzed to record the performance of the impact of stress on longevity. The flies were subjected to various stressors like temperature, solvent fumes (ethanol and acetone fumes), desiccation, oxidation, as well as starvation. The report has revealed that females are more tolerant than males in both the races; flies exposed to starvation have shown significantly increased longevity in both the races. Interestingly, the derived race *D. n. albomicans* is significantly prone to be more resistant to variable stressors with increased longevity than ancestral race *D. n. nasuta*. Thus the derived race is more fit than ancestral race.

Key words: *Drosophila nasuta nasuta* · *Drosophila nasuta albomicans* · S tressors · Temperature · Longevity

INTRODUCTION

A variety of factors may affect stress tolerance of the organisms such as physiological and behavioral changes. Climatic changes may be met by e.g. physiological hardening processes, coma or production of metabolites making the organism tolerate temperature extremes [1, 2]. Diet restriction or mild starvation can increase longevity as well as tolerance to stressors such as heat stress [3, 4] demonstrating the complexity of nutrient acquisition and utilization of the organisms. Behaviorally, organisms may respond to stress by migration, use of refuges or an altered and more favorable nutrient intake to meet changed energy expenditures [5]. *Drosophila melanogaster* is often used as a model organism in studies of physiological and evolutionary responses to various forms of stress [6, 7].

The physiological changes in turn affect life history and fitness traits such as fecundity, longevity and stress resistance. Life-history evolution in *Drosophila* has been extensively studied and laboratory selection studies on *D. melanogaster* have, in particular, yielded much insight into the various tradeoffs surrounding major life-history traits such as preadult development time, age-specific female fecundity and adult lifespan [8]. Life history and stress tolerance are generally related to habitat. Environmental stress plays an important role in the maintenance of genetic variation [9] and in evolution [10]. Several studies support the notion of a cost of reproduction in terms of increased mortality and, hence, decreased adult lifespan in *D. melanogaster* [11-14].

The *nasuta* subgroup of the *immigrans* species group of *Drosophila* has attracted the attention of taxonomists, cytogeneticists, biochemists, molecular biologists and evolutionary biologists. This subgroup of *Drosophila* has certain evolutionary peculiarities, which include little morphological differentiation among species despite their distribution over an enormous territory and the ability of species to intercross in the laboratory, often producing fertile offspring and substantial chromosomal evolution in the hybrids. These features make this subgroup a potent system to study the genetics of early

Corresponding Author: B.P. Harini, Drosophila Culture Laboratory, Department of Zoology, Jnana Bharathi, Bangalore University, Bangalore - 560 056, India.

stages of speciation in *Drosophila*. *D. nasuta nasuta* (2n = 8) and *D. n. albomicans* (2n = 6) are a pair of sibling allopatric chromosomal races of the *nasuta* subgroup of *Drosophila*. The cytological distinctness of these two races has been extensively studied [15]. *D.n. nasuta* is considered as the ancestor race and *D. n. albomicans* as the derived race [16]. In view of this, the study was undertaken to compare the influences of different types of stress (temperature, solvent fumes (ethanol and acetone fumes), desiccation, oxidation, as well as starvation) on longevity and to record the subtle evolutionary divergence of the two races on exposure to different stress and its impact on longevity.

MATERIALS AND METHODS

The *Drosophila* stocks used in the present experiment were *Drosophila nasuta nasuta* (Coorg, India) and *Drosophila nasuta albomicans* (Okinawar, Texas Collection, USA, 3045.11). The stocks were obtained from the *Drosophila* stocks centre, Mysore, India. The stocks were accessed to investigate the impact of various stresses on longevity. The experimental assays were conducted following the protocol Harshman *et al.* [17] with slight modifications.

The fly stocks used in the present study were reared on standard wheat cream agar medium in an uncrowded condition at variable temperatures with a relative humidity of 70%. About 100 to 150 flies were collected and were exposed to each of the variable temperature (18°C, 22°C and an ambient laboratory temperature ranging from 22°C to 26°C Room Temperature) and different stressors namely, Ethanol, Acetone, Desiccation, Starvation and Paraquat for about 5 to 6 generations to stabilize and breed true. The following assays were conducted to test the resistance of these two allopatric races to test the impact of stress on longevity.

Assays for Resistance to Solvent Fumes: Adult flies after ecolsion were separated by sex under light ether anesthesia prior to the beginning of the assay. Then the flies were transferred to empty vials plugged with cotton. Ten males and females were placed in empty 8-dr vials with cotton plug. About 10-15 replications were maintained and tested simultaneously for resistance to solvent fumes. A volume of 150 ml of 100% ethanol and acetone was placed in the dessicator. The cotton plugged vials with flies were placed in a sealed dessicator and were exposed to 18°C, 22°C and 22°C to 26°C for about 5h separately. At the end of this exposure, the seal was broken and all the flies were transferred to recovery vials with fresh food vial seeded with yeast grains before determining the number of surviving individuals. Likewise, they were successively transferred to fresh food vials every alternate day until all the flies were found dead. The mortality was recorded daily for the flies exposed to both the solvent fumes.

Assay for Resistance to Desiccation: Resistance to desiccation was accessed for about 6-8 generation. The general procedure was, for a specified period, to keep flies in an empty vial away from water source. Ten males and 10 females were placed in desiccators. About 10-15 replications of each stock were kept in desiccators exposed to variable temperature (18°C, 22°C and RT) for about 5h without access to water. After the desiccation period all the individuals were transferred to vials with fresh food for a recovery period and then they were successfully transferred to fresh food vials seeded with yeast every alternate dates and were recorded for the number of survivals.

Oxidation Stress Assay: A 30 mM solution of methyl viologen (paraquat) was prepared in 15% sucrose solution. Filter paper (whatman1,) discs were cut to match the inside diameter of 8-dr vials. A total of eight discs were placed in each empty vial and then the discs were wetted with 0.5 ml of methyl viologen-sugar solution. About 10 to 15 replicates having 10 flies' in each vial were simultaneously tested for both males and females by exposing flies with paraquat for 5 h at 18°C, 22°C and RT separately. To retard evaporation and to hold flies in the vials foam stoppers were used. After the treatment all the flies were transferred to vials containing fresh medium, likewise the flies were transferred every alternate days and the number of surviving individuals was recorded.

Starvation Assay: About 10-15 replications of both males and females flies of *D. n. nasuta* and *D. n. albomican* stocks were cultured under uncrowded conditions were transferred to empty vials at a density of 10 flies in each vial. The vials were plugged with cotton saturated with water was kept at 18°C, 22°C and RT for 24 h without access to the food source. Then the males and females were transferred to fresh food vials containing yeast granules likewise successive changes were made and recorded for the mortality.

It was taken care that every alternate days at approximately the same time, the flies from the vials of the previous day was removed and they were replaced to the

fresh vial. The respective vials were maintained at 18°C, 22°C and RT. The mortality was recorded in each vial until no flies remained alive.

Statistical Analysis: One-way ANOVA was performed for stresses versus longevity for both the races of *immigrans* subgroup of *Drosophila*. Duncan's multiple range test (DMRT) was performed to ascertain the differences. All analyses were performed using the statistical presentation system software package SPSS 15.0 for MS Windows.

RESULTS

Observations Recorded for *Drosophila nasuta Nasuta:* Table 1 reveals that the Control flies reared at different temperature have shown significantly increased mean longevity than the flies exposed to different stressors in *D. n. nasuta*. Females have shown increased longevity than males under all the variable stressors exposed to different temperature except desiccation. In desiccation, males have shown increased mean longevity at 18°C but the differences are insignificant. The comparison among the variable stress indicates (Ethanol, Acetone, Desiccation, Starvation, Paraquat) the flies exposed to starvation and desiccation stress have significantly increased and decreased longevity at different temperature regimes. The longevity of male and female flies has shown higher when exposed to 18°C followed by 22°C and RT.

The analysis of variance have shown significant differences for all the trails (Temp Vs Stressors) with P>0.001. According to Dunccan's multiple range test the differences are significant between 18°C and 22°C and also between 18°C and RT for all the stress variables. While the males and females of control, females exposed to ethanol and the males exposed to acetone and starvation have shown significant differences between 22°C and RT. Longevity at 18°C was the highest and at RT was the lowest, while it was intermediate at 22°C. The order of ranking according to DMRT for stress wise comparison was 18°C > 22°C > RT;The sequential order of stressors for *Drosophila nasuta nasuta* was Control < Starvation< Paraquat< Acetone fumes < Ethanol fumes< Desiccation.

Observations Recorded for *Drosophila nasuta Albomicans*: Table 2 reveals that the Control flies reared at different temperature have shown significantly increased mean longevity than the flies exposed different stressors in *D. n. albomicans*. The flies exposed to paraquat at 22°C have shown that the female lives significantly longer than males comparing with all trails exposed to different temperature and variable stressors. The males and females of *D. n. albomicans* have shown greater longevity under starvation condition while it is lowest when exposed to desiccation stress. The longevity of male and female flies has shown higher when exposed to 18°C followed by 22°C and RT.

Table 1: Mean longevity of *Drosophila nasuta nasuta* exposed to various stressors at three different temperature

Stress→	Control		Ethanol		Acetone		Desiccation		Starvation		Paraquat	
Temp↓	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
18°C	43.06±2.10	49.06±2.15	24.00±0.71	27.82±0.81	27.60±0.63	30.04±1.20	21.13±0.45	19.51±0.39	42.14±1.98	45.02±2.31	36.22±1.53	39.89±1.66
22°C	41.02±2.23	45.80±2.26	21.22±0.86	23.17±0.97	24.55±1.13	27.25±1.21	16.25±0.40	16.91±0.47	37.92±2.40	41.13±2.51	33.00±1.76	35.06±1.83
RT*	34.02±1.25	36.00±1.76	19.20±0.99	20.48±1.15	20.92±0.98	25.81±1.36	15.17±0.52	17.15±0.11	35.80±0.80	39.88±1.58	34.04±1.96	35.02±2.03
ANOVA	F=2.911	F=2.80	F=2.80	F=2.801	F=3.200	F=3.202	F=3.413	F=2.671	F=3.931	F=3.671	F=3.060	F=3.060
	Df=2,297	Df=2,297	Df=2,297	Df=2,297	Df=2,297	Df=2,297	Df=2,297	Df=2,297	Df=2,297	Df=2,297	Df=2,297	Df=2,297
	P<0.0001	P<0.002	P<0.002	P<0.001	P<0.001	P<0.01	P<0.001	P<0.021	P<0.001	P<0.001	P<0.001	P<0.001
DMRT	18°C/22°C	18°C/22°C	18°C/22°C	18°C/22°C	18°C/22°C	18°C/22°C	18°C/22°C	18°C/22°C	18°C/22°C	18°C/22°C	18°C/22°C	18°C/22°C
	18°C/RT	18°C/RT	18°C/RT	18°C/RT	18°C/RT	18°C/RT	18°C/RT	18°C/RT	18°C/RT	18°C/RT	18°C/RT	18°C/RT
	22°C/RT	22°C/RT		22°C/RT	22°C/RT				22°C/RT			

*Note: RT means Ambient laboratory temperature ranging from 22°C to 26°C

Table 2: Mean longevity of *Drosophila nasuta albomicans* exposed to various stressors at three different temperatures

Stress→	Control		Ethanol		Acetone		Desiccation		Starvation		Paraquat	
Temp↓	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
18°C	45.16±2.05	49.15±2.17	30.61±0.91	33.42±1.19	28.80±2.10	35.08±2.83	27.23±1.61	43.33±2.62	37.18±2.51	44.40±2.98	36.18±1.73	40.05±2.31
22°C	43.12±2.61	44.10±2.30	28.01±1.06	31.03±2.09	30.02±1.24	33.83±1.41	19.25±0.99	21.17±1.96	39.63±2.67	42.18±2.89	38.02±2.56	35.06±1.83
RT*	34.81±2.25	39.82±2.77	23.82±0.95	29.99±2.15	27.66±1.59	28.13±2.61	15.16±0.87	18.66±1.11	36.90±2.80	39.91±2.63	34.08±2.06	36.13±2.79
ANOVA	F=2.030	F=2.506	F=2.111	F=2.932	F=3.600	F=3.903	F=3.314	F=2.781	F=3.321	F=3.764	F=3.621	F=3.453
	Df=2,297	Df=2,297	Df=2,297	Df=2,297	Df=2,297	Df=2,297	Df=2,297	Df=2,297	Df=2,297	Df=2,297	Df=2,297	Df=2,297
	P<0.0001	P<0.0001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.002	P<0.001	P<0.001	P<0.0001
DMRT	18°C/22°C	18°C/22°C	18°C/22°C	18°C/22°C	18°C/22°C	18°C/22°C	18°C/22°C	18°C/22°C	18°C/22°C	18°C/22°C	18°C/22°C	18°C/22°C
	18°C/RT	18°C/RT	18°C/RT	18°C/RT	18°C/RT	18°C/RT	18°C/RT	18°C/RT	18°C/RT	18°C/RT	18°C/RT	18°C/RT
	22°C/RT	22°C/RT	22°C/RT				22°C/RT	22°C/RT		22°C/RT	22°C/RT	

*Note: RT means Ambient laboratory temperature ranging from 22°C to 26°C

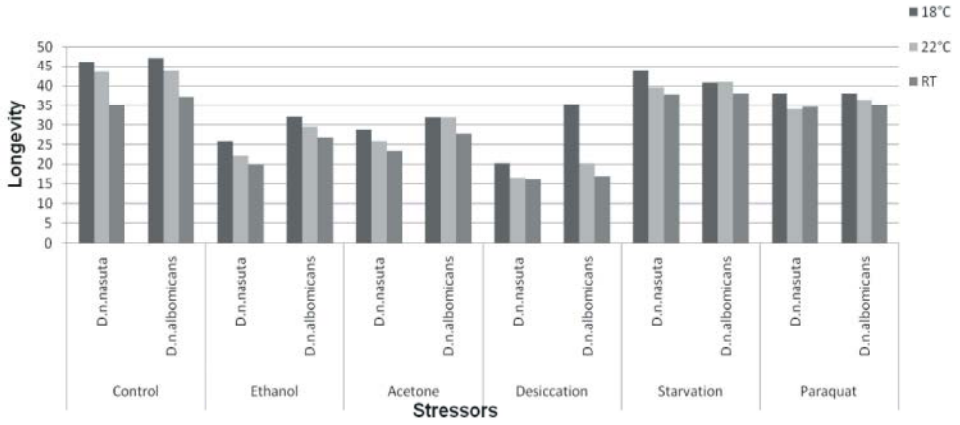

Fig 1: Mean longevity of *Drosophila nasuta nasuta* and *Drosophila nasuta albomicans* exposed to variable stressors at three different temperatures

The analysis of variance have shown significant differences for all the trails (Temperature Vs Stressors) with P>0.001. According to Duncan's multiple range tests the differences are significant between 18°C and 22°C and also between 18°C and RT for all the stress variables. 18°C> 22°C> RT; The order of ranking according to DMRT for stress wise Comparison follows Control < Starvation< Paraquat< Acetone fumes < Ethanol fumes< Desiccatio for *D. nasuta albomicans*.

Figure 1 depicts *D. n. albomicans* have shown increased values for longevity even though they are exposed to variable stressrs at different temperature regimes when compared to the ancestral race of *D. n. nasuta*. Thus the derived race is more potent than the ancestral race *D. n. nasuta*. Interestingly, Females have shown increased longevity than males under all the variable stressors exposed to different temperature expect in desiccation. In desiccation, males have shown increased mean longevity at 18°C but the differences are insignificant. The flies exposed to 18°C live longer than the flies cultured at 22°C or at room temperature. Except starvation the other stressors has led significant decrease in longevity in both the races. Interestingly, the derived race *D. n. albomicans* is significantly prone to be more resistance to variable stressors with increased longevity than ancestral race *D. n. nasuta*. Thus the derived race is more fit than ancestral race in terms of resistance to stress.

DISCUSSION

In the comparative plant and animal literature, there is an abundance of evidence for multiple-stress resistance. A genetic basis for multiple-stress resistance is indicated by strain comparisons, family-based genetic correlation analyses and selection experiments [18]. Selection experiments using *Drosophila* have proven particularly useful in this regard. For example, Hoffmann and Parsons [19,20] selected for desiccation resistance using *D. melanogaster* and documented correlated resistance to a range of other stresses. Selection for ethanol tolerance using different species of *Drosophila* often results in correlated tolerance, or resistance, to other stressors [21]. The relationship between environmental stress, neurodegeneration and aging is an important investigatory nexus. In this area, ongoing research relates stress, glucocorticoids and damage to the hippocampus [22].

Variations of lifespan within natural populations are partly attributable to both genetic and environmental effects [23]. It has been reported that at least two types of environmental stress factors, extreme temperature and poor nutrition, consistently increase the phenotypic plasticity [24]. That tolerance to desiccation was highest in flies developed on the protein-enriched medium is not what would be expected based on the results from studies by Parkash *et al*. [25] where desiccation tolerance and lipid accumulation is shown to be positively correlated. It is however possible that the metabolic end-product from protein metabolization, uric acid, had a protecting effect on the increasing osmotic pressure during desiccation by reducing the water loss from cells [26]. One set of *D. melanogaster* lines selected for increased resistance to starvation and showing increased resistance to a diversity of stresses, did not show a correlated change in longevity [7]. Moreover, one set of long-lived lines did not show a substantial increase in desiccation and starvation resistance [27] and in another set of lines there

was a correlated change in stress resistance under selection for reduced longevity but not increased longevity [28].

Variation in stress-related traits in insects and other organisms has been widely studied because it underlies the ability of insects to adapt and counter the effects of changing climatic conditions. For instance, in *Drosophila* a high level of desiccation resistance is associated with adaptation to arid habitats while a high level of cold resistance is linked to adaptation to high latitudes [29]. The relevant *Drosophila* data are based almost exclusively on *D. melanogaster*. Long-lived selection lines of this species are often relatively more resistant to starvation and desiccation stress [30].

The course of the present study is to evaluate whether the ancestral or the derived race is more resistance to stress in terms of longevity. The study reveals that both the males and females of derived race i.e. *D. albomicans* have explored significantly more values for stress resistance versus longevity in control and as well as in treated flies. The data opines with the earlier report that *D. nasuta albomicans* have significantly increased values for mating activity, productivity and longevity even when exposed to different temperature and light regimes when compared to *D. n. nasuta* [31, 32]. Both males and females of *D. n. nasuta* as well *D. n. albomicans* have shown increased and decreased resistance to starvation, desiccation, ethanol fumes, acetone fumes and paraquat. However, the males exposed to desiccation and paraquat at 18°C and 22°C have shown increased mean longevity respectively, but the differences are insignificant. While in all the other experimental trails exposed to different temperature and variable stressors have shown that the female lives significantly longer than males. Quantitative aspects of lifespan and its correlates are well categorized in Drosophila [33]. There are reports that females of *Drosophila* had significantly increased lifespan than males, with a few exceptions [11] suggested that because of their distinctive roles in reproduction, females and males are selected towards different optimal phenotypes.

On exposure to paraquat *D. n. albomicans* has increased longevity than *D. n. nasuta* but the differences are insignificant. Paraquat resistance increased in the selected lines and their was evidence for the upregulation of a variety of anti oxidant ezymes [34]. Resistance to paraquat is significant in the context of the free radical theory of ageing [35]. The flies of the two races under study have experienced increased longevity at lower temperature (18°C) and decreased longevity at fluctuating room temperature and intermediate at 22°C, in spite of this the derived race *D. n. albomicans* have flourished with significantly higher values for longevity than the ancestral *D. n. nasuta*. This suggests that the derived race live longer than the ancestral race with better lifespan even though they are exposed to different temperature regimes. Therefore, the effect of temperature is an important facet which determines longevity. One can surmise that the effect of various stresses on longevity reflects the action of the extent of resistance found to be significantly high in the derived race in an evolutionary process.

Therefore, we put forth that the derived race live better and longer than ancestral race. This observation is yet another important avenue to quantify subtle evolutionary divergence and also indicates that desiccation and starvation resistance vary markedly between two races and it is evident there is a relationship between stress resistance and longevity. Thereby, rigorous comparative analyses between races needed to address if heterogeneous outcomes are common and also whether correlations between stress resistance traits and life history characters act as evolutionary constraints.

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